

1 WHAT IS CLAIMED IS:

1 1. A method for the vitrification of biological materials, said method comprising the  
2 steps of:

3 (a) suspending the biological material in a cryoprotective equilibration medium,  
4 having a concentration of cryoprotectant(s) below that sufficient to protect against  
5 ice formation to the glass transition temperature of the cryoprotective equilibration  
6 medium; A

7 (b) rinsing the equilibrated biological material with a vitrification solution, the  
8 vitrification medium having a concentration of cryoprotectant(s) sufficient to  
9 protect against ice formation to the glass transition temperature of the vitrification  
10 medium; and

11 (c) dropping the vitrification solution-rinsed biological material in microdroplets  
12 of vitrification solution onto a solid surface with good heat conductivity having  
13 been previously cooled down to about -150°C to about -180°C.

1 2. The method of claim 1 wherein the biological material is a cell.

1 3. The method of claim 1 wherein the biological material is an oocyte. B

1 4. The method of claim 1 wherein the biological material is an embryo.

1 5. An improved method for cryopreserving biological material suspended in a  
2 vitrification solution, wherein the improvement comprises contacting microdroplets of the  
3 vitrification solution containing the biological material with a solid surface having a  
4 temperature of about -150°C to about -180°C, said surface having a good heat  
5 conductivity. A

1 6. The method of claim 5 wherein the biological material is a cell.

1 7. The method of claim 5 wherein the biological material is an oocyte.

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1 8. The method of claim 5 wherein the biological material is an embryo.

Sub AA

2 9. An improved method for cryopreserving biological material suspended in a  
3 vitrification solution, wherein the improvement comprises contacting microdroplets of the  
4 vitrification solution containing the biological material with a solid surface having a  
5 temperature of about -150°C to about -180°C, said surface having a thermal conductivity at  
6 20°C of greater than about 10 W/(m-k).

Sub AB

7 10. A method for the vitrification of oocytes, said method comprising the steps of:

8 (a) suspending the oocytes in a cryoprotective equilibration medium, having a  
9 concentration of cryoprotectant(s) below that sufficient to protect against ice  
10 formation to the glass transition temperature of the cryoprotective equilibration  
11 medium;

12 (b) rinsing the equilibrated oocytes with a vitrification solution, the vitrification  
13 medium having a concentration of cryoprotectant(s) sufficient to protect against ice  
14 formation to the glass transition temperature of the vitrification medium; and

15 (c) dropping the vitrification solution-rinsed oocytes in microdroplets of  
16 vitrification solution onto a solid surface with good heat conductivity having been  
17 previous cooled down to about -150°C to about -180°C.

18 11. An improved method of transferring nuclear DNA from a donor cell to an  
19 enucleated oocyte, said improvement comprising the step of introducing the nuclear  
20 material of the donor cell into an enucleated oocyte derived from an oocyte vitrified by the  
21 method of claim 10.

22 12. An oocyte vitrified by the method of claim 10.

23 13. An embryo developed from the oocyte of claim 12.

24 14. A fetus developed from the oocyte of claim 12.

25 15. An animal developed from the oocyte of claim 12.

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- 1 16. A cell line developed from the embryo of claim 13.
- 1 17. A cell line developed from the fetus of claim 14.
- 1 18. A cell line developed from the animal of claim 15.
- Sub A6  
2 19. An improved method for cryopreserving oocytes suspended in a vitrification  
3 solution, wherein the improvement comprises contacting microdroplets of the vitrification  
4 solution containing the oocytes with a solid surface having a temperature of about -150°C  
5 to about -180°C, said surface having a thermal conductivity 20°C of greater than about 10  
W/(m-k).
- 1 20. An oocyte vitrified by the method of claim 19.
- 1 21. An embryo developed from the oocyte of claim 20.
- 1 22. A fetus developed from the oocyte of claim 20.
- 1 23. An animal developed from the oocyte of claim 20.
- 1 24. A cell line developed from the embryo of claim 21.
- 1 25. A cell line developed from the fetus of claim 22.
- 1 26. A cell line developed from the animal of claim 23.
- 1 27. An improved method for the parthenogenetic development of vitrified oocytes  
2 cultured in a KSOM plus BSA culture system, the improvement comprising co-culturing  
3 with cumulus-cells.

Add A7 > Add C1 >

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